TITLE: N-NITROSOPROLINE, AN INDICATOR FOR N-NITROSATION OF AMINES IN TOBACCO PRODUCTS

AUTHORS: J. C. Scott*, K. D. Brunnemann, and D. Hoffmann

AFFILIATION: American Health Foundation,
Valhalla, NY 10595

ABSTRACT: This study examines whether N-nitrosoproline (NPRO) can serve as a reliable indicator of the extent to which amines can be N-nitrosated to volatile, non-volatile and tobacco-specific N-nitrosamines during processing and smoking of tobacco. Free proline is found in green leaves up to 2,000 ppm and in processed tobacco up to 4,000 ppm. It is amenable to N-nitrosation. Analytical procedures begin with extraction of tobacco with dilute sulfuric acid in the presence of ascorbic acid. ^{14}C-NPRO is added as an internal standard for quantitative analyses. The filtrate of tobacco extract is partitioned with ethyl acetate and the organic material is chromatographed on silica gel. The concentrated NPRO fraction is then methylated and analyzed by GC-TEA. Proline itself is determined as NPRO, following the nitrosation of tobacco (^{14}C-proline serves as internal standard). Cigarette tobacco contains up to 2 ppm and fine cut chewing tobacco contains up to 35 ppm of NPRO. In the case of cigarette tobacco, less than 1% of the free proline is nitrosated whereas up to 40% of the free proline is nitrosated in chewing tobacco. It will be shown that the biologically inactive NPRO can be used as an indicator for the degree of N-nitrosation of volatile amines and tobacco alkaloids. The absolute values for NPRO depend on the proline and nitrate concentrations of tobacco as well as the method of tobacco processing. Analytical data on the transfer rate, formation, and fate of NPRO during smoking will also be presented.

REVIEW: N-nitrosoproline (NPRO) is non-genotoxic and appears unchanged in the urine of experimental animals. It is of interest to the authors as an indicator of amine nitrosation. Free proline, as well as being found in green and processed tobaccos, is a major amino acid in bright leaf. Procedures described included the use of ^{14}C-labeled materials as tracers, common to most reports from the A.H.F. group. Characterization of the NPRO isolated from tobacco included peroxide oxidation wherein one-half was converted to the nitro compound (nitramine). This gave a second GC/TEA peak. Subsequent treatment of either the known or the isolate with HBr/HOAc caused de-nitrosation with the disappearance of the corresponding peak from each chromatogram. The nitramine was unchanged. Initially, no NPRO was found in smoke, but, when ^{14}C-spiked cigarettes were smoked, it was detected with 0.14% transfer. Concurrently, the equivalent of 0.073% was found as N-nitrosopyrollidine (NPYR). This indicates that N-nitrosoproline, although not a major contributor, does yield N-nitroso-pyrollidine in smoke. The possibility of proline itself being a more direct precursor during smoking was not mentioned (i.e., by simultaneous or sequential N-nitrosation and decarboxylation); however, it is included in a pre-publication manuscript as occurring "to a limited degree." As indicated in the abstract, processing affected the final level of NPRO in tobacco, with flue-cured the lowest and fine-cut snuff the highest. This parallels earlier results on the tobacco-specific nitrosamines reported by the same group.

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